DIAGNOSIS OF WBCS

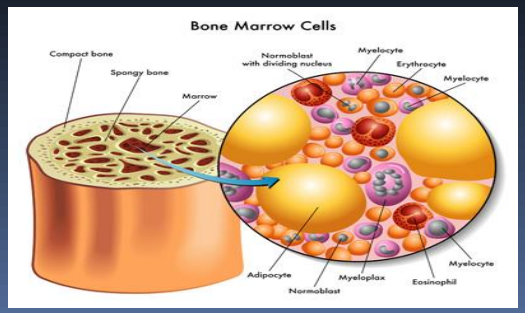
Blood Cell Origin and Production

All WBCs

(except lypmphocytes)

organs

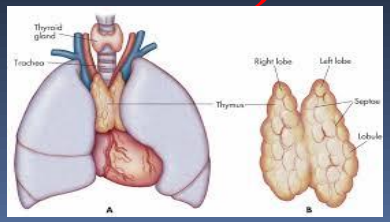
Bone marrow



Lymphocytes

1ry and 2ry lymphoid

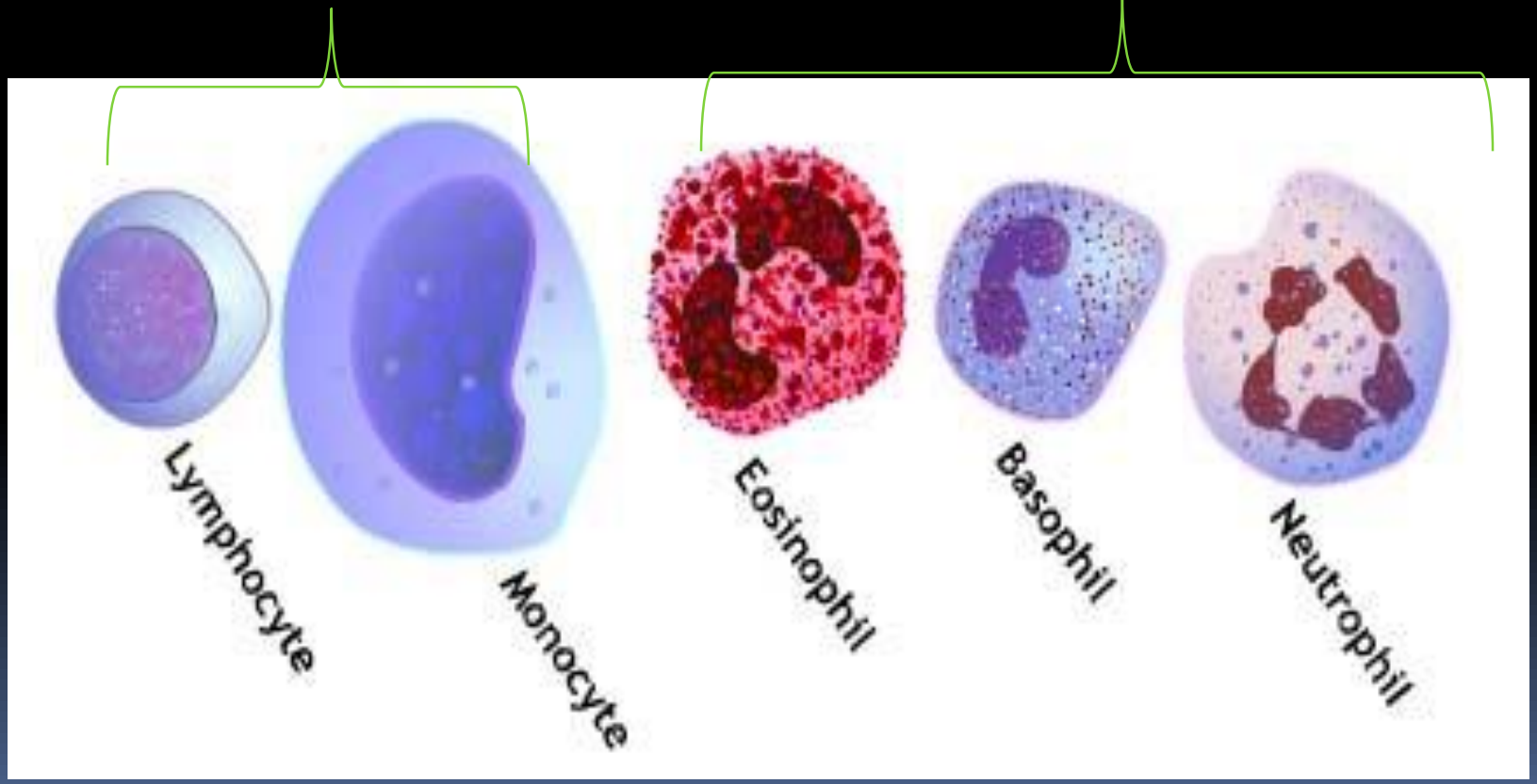
(thymus gland and lymph nodes)



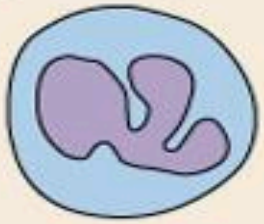
- In normal adults: 4,000-11,000/mm3
- In children: slightly higher
- **WBC disorders:**
  - Quantitative (abnormal number)
  - Qualitative (poorly functioning cells)

Types of WBC's

Agranulocytes **Granulocytes**



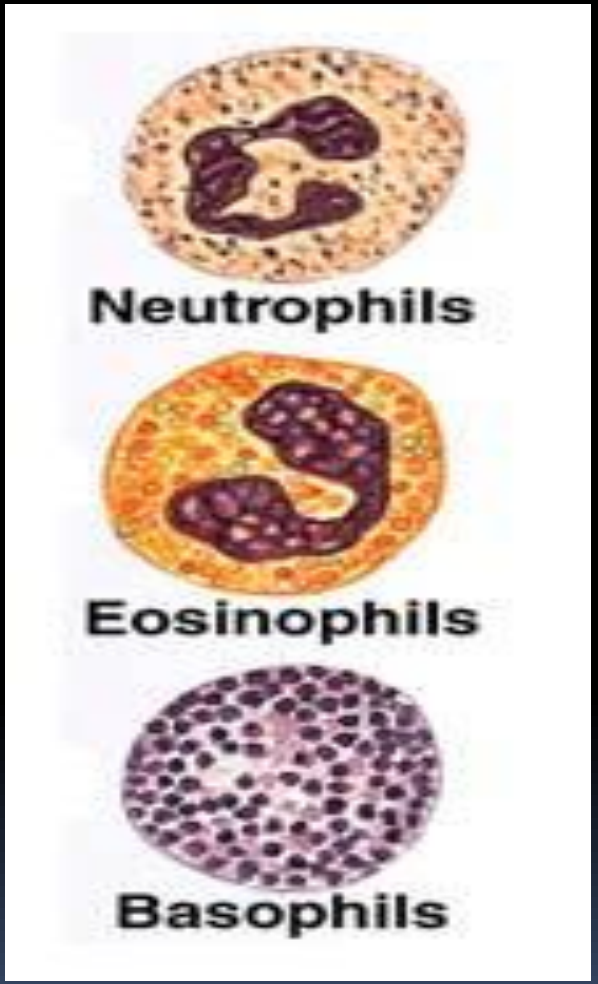
Leukocytes

Leukocyte group	Subgroup	Function
granulocytes	neutrophils	destroy small organisms
	basophils	secrete histamine, mediating inflammatory response, and platelet activating factor
	eosinophils	destroy parasites allergic reaction
lymphocytes	B lymphocytes	synthesize antibody
	T lymphocytes	participate in the specific immune response
monocytes	macrophages	destroy invading organisms

Granulocytes

2 types of granules in their cytoplasm:

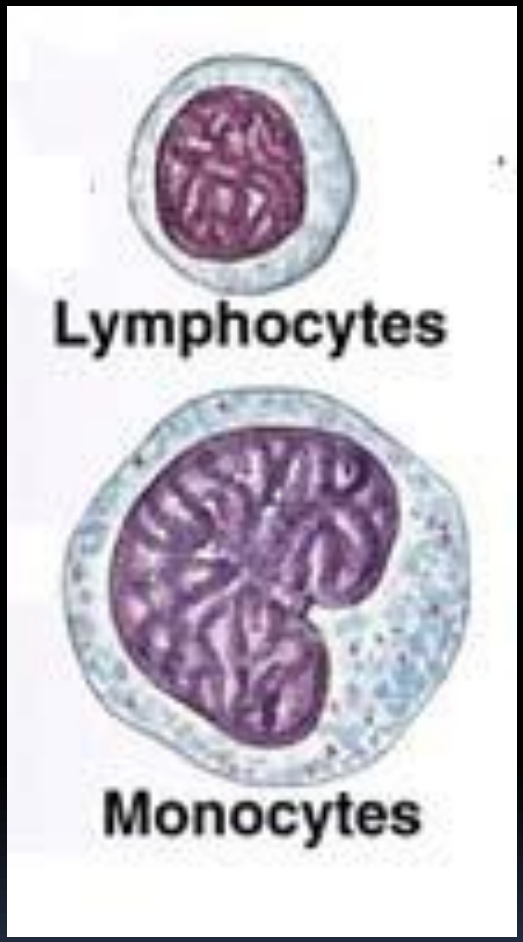
- **Specific granules (specific functions)**
- **Azurophilic granules (lysosomes)**



Agranulocytes:

Do not have specific granules,

but they do contain azurophilic granules in their cytoplasm



“Bands”

Less mature form of neurophils

“Left shift”

Increase in band cells

Leukocytosis

It is the increase in number of white blood cells more than 11000 /mm3

Causes

- **Physiologic**
  - Extreme Temp
  - Exercise
  - Stress
- **Pathologic**
  - Infection
  - corticsteroids
- Leukemia

Decrease in total count

- **Decreased Production**
  - Bone marrow depression

  (Aplastic A, Cytotoxic drug, bone metastasis)
- **Increased Destruction**
  - Hypersplenism
  - Autoantibodies
  - Bacterial infection
  - Viral Infection HIV

Differential WBC Count

- **Relative**
  - % of each cell type in relation to the total
- **Absolute**
  - True cell count for each type

Differential Count:

	Absolute	%
Neutrophils Segmented Bands	0-2000/mm3 3000-6000/mm3	60-70%
Basophils	0-100 /mm3	0-1%
Eosinophils	100-700/mm3	1-3%
Lymphocytes	1000-4000 mm3	20-35%
Monocytes	100-900/mm3	2-6%

Changes in Neutrophils Count:

Increase (Neutrophilia)
Acute bacterial infection Sterile inflammation (as that associated with tissue necrosis in cases of burns and myocardial infarction) Myeloid(myelogenous) leukemia

Decrease (neutropenia)

Decreased production as in

- Aplastic anemia
- Bone marrow depression after irradiation.
- Cytotoxic drug therapy

Increase destruction

- Idiopathic neutropenia
- Hypersplenism
- Peripheral use as in overwhelming bacterial, fungal or rickettsial infections.

Changes in Eosinophils Count:

Increase (eosinophilia)
• Allergic disorders • Parasitic infestations • Drug reactions • Chronic myelogenous leukaemia

Decrease (eosinopenia)

- Aplastic anemia
- Typhoid

Changes in Lymphocytes Count:

Increase (Lymphocytosis)
Chronic infections Lymphocytic leukaenia Some viral infections e.g. mumps

Decrease (Lymphocytopenia)

- Aplastic anemia
- Immunodeficiency disorders
  e.g. HIV infection (AIDS)

Changes in Monocytes Count :

Increase (monocytosis)
• Chronic infections (e.g. TB) • Bacterial endocarditis • Malaria • Monocytic leukaemia

Decrease (monocytopenia)

- Aplastic anemia

Basic mechanism of hemostasis

A. Vascular Phase
B. Platelet Phase
C. Coagulation Phase
D. Fibrinolytic Phase

- **A] Vascular Phase**

Tissue injury vasoconstriction

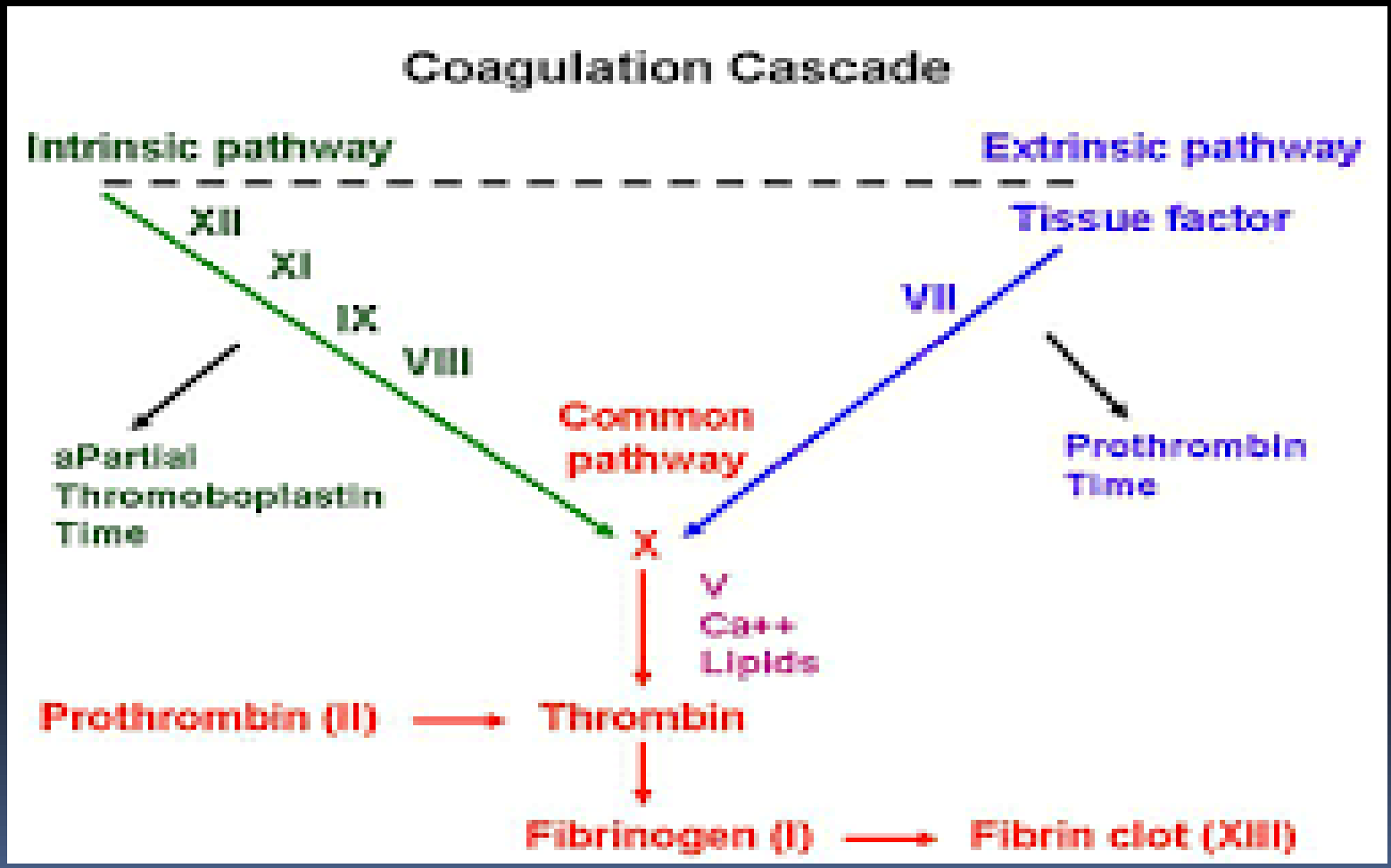
Stimulated by:

Serotonin

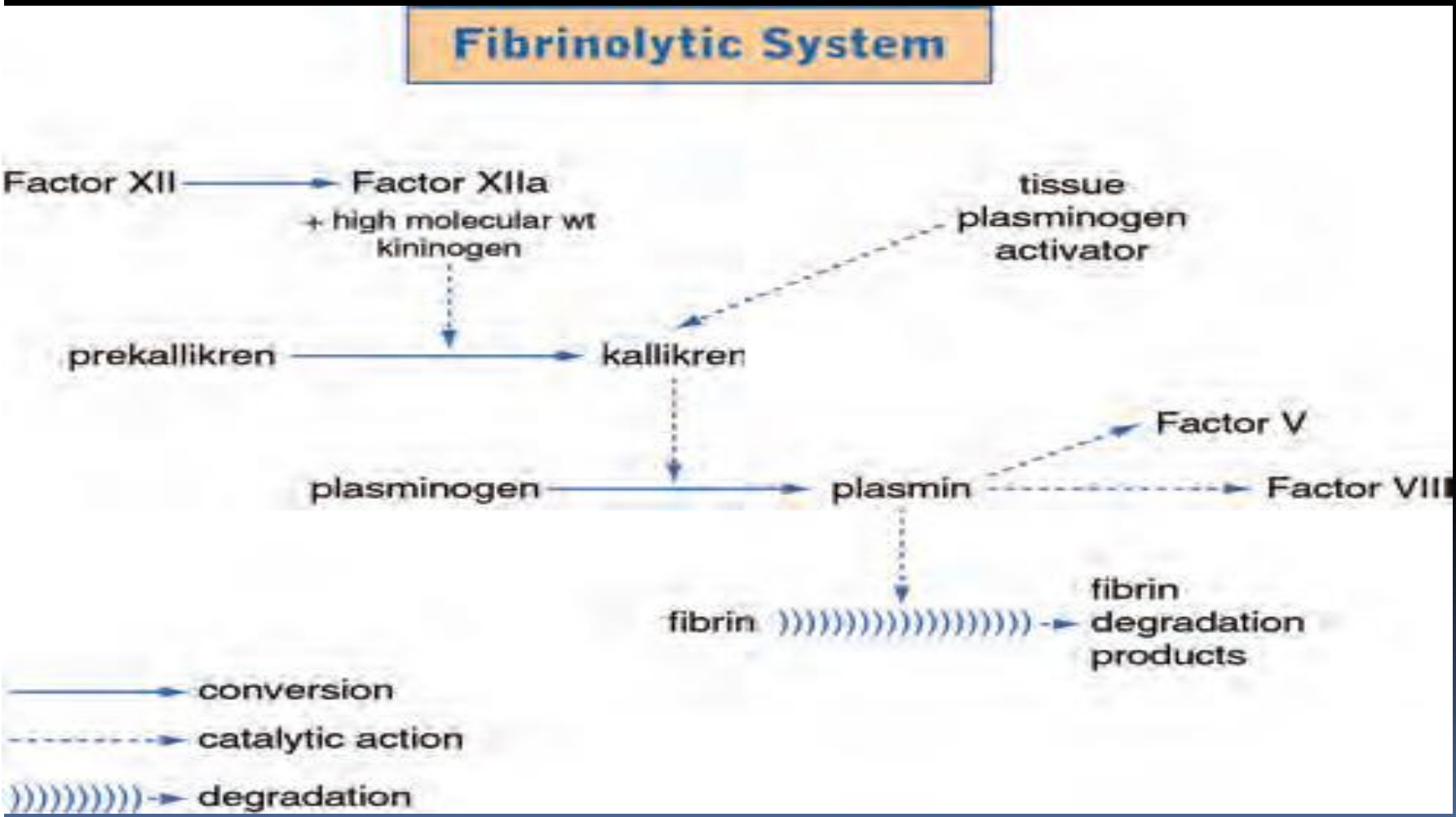
Histamine

Prostaglandins

C] Coagulation Phase



D] Fibrinolytic Phase



Tests for bleeding tendency

A] Vascular Phase

Increased blood vessels fragility **"Hess test"**

B] Platelet Phase

Platelet deficiency or dysfunction

Tested for by:

a) Platelet count

b) Bleeding time

c) Clot retraction

d) Specific tests for platelet function.

C] Coagulation Phase

a) Clotting time

b) Prothrombin time (PT)

c) Partial thromboplastin time (PTT) & activated partial thromboplastin time (APTT) .

d) Coagulation factors assays.

A]Testing cappillary function

Hess Test (Tourniquet test):

> 10 petechiae means increased capillary fragility

B] Testing Platelet deficiency or dysfunction

a) **Platelet count**

- **Normal platelet count:** 150,000-500,000/mm3.

- **Thrombocytopenia** (<150,000/mm3)

  20,000-50,000 ➡ Bleeding only with trauma and surgery

  Less than 20,000 ➡ spontaneous bleeding

  Less than 5,000: ➡ profuse spontaneous hemorrhage

- **Thrombocytosis or thrombocythemia**

  (may reach 1,000,000/mm3)

  - Bleeding occurs with thrombocytosis due to abnormal function despite the increase in number.

b) Bleeding time:

it measures directly the cessation of bleeding.

i) Duke method: in which bleeding from a pricked ear lobe is measured

Normal bleeding time by this method is **2-4 min**.

ii) Ivy method

A Blood is blotted every 30 seconds from the wound with filter paper until bleeding stops

Bleeding Time is then Measured by:

$$\text{Bleeding time} = \frac{\text{no. of blots}}{2}$$

Normal bleeding time by this method **= 2 - 9** minutes (average 5 minutes).

Bleeding time is prolonged in:

1. Thrombocytopenia
2. Thrombocytosis
3. Thromboasthenia (deficient platelet aggregation: Glanzmann's disease).
4. Thrombocytopathy **(deficient platelet adherence: Bernard-Soulier syndrome).**
5. Aspirin therapy (and other non steroidal anti-inflammatory drugs): these drugs inhibit platelet aggregation and their release reaction.
6. Uremia.

Specific Tests for Platelets Function:

a) **Platelet Adherence**:

- Fresh blood is passed over glass beads (to which normal platelets adhere).
- Not less than 20% of platelets should be found adherent to the beads.

This no. is decreased in:

1. Thrombocytopathy
2. Thrombocythemia
3. Uremia

b) Platelet Aggregation: (using aggregometer).

ADP, Collagen, thrombin and epinephrine cause aggregation of normal platelets.

- Decrease in:
- Thromboasthenia
- aspirin therapy.

c) Platelet Factor 3 Activity.

- Platelet factor 3 is produced by platelets during the release reaction and helps in blood coagulation.

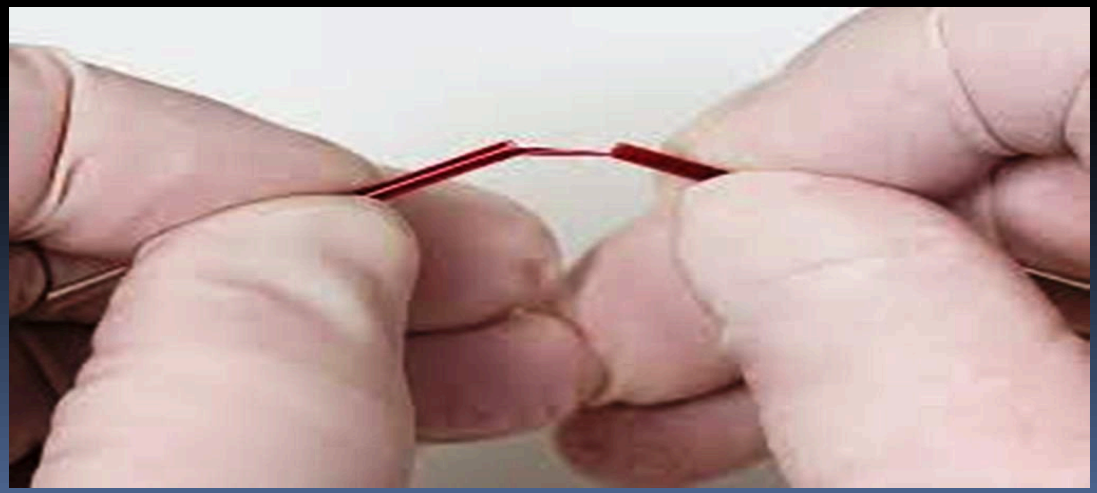
d) Detection of anti platelet antibodies (autoimmune thrombocytopenia).

C]Testing the Clotting Factors:

1) **Clotting (coagulation) time (Lee- White time)**

"The time taken by freshly collected blood to form a firm clot"

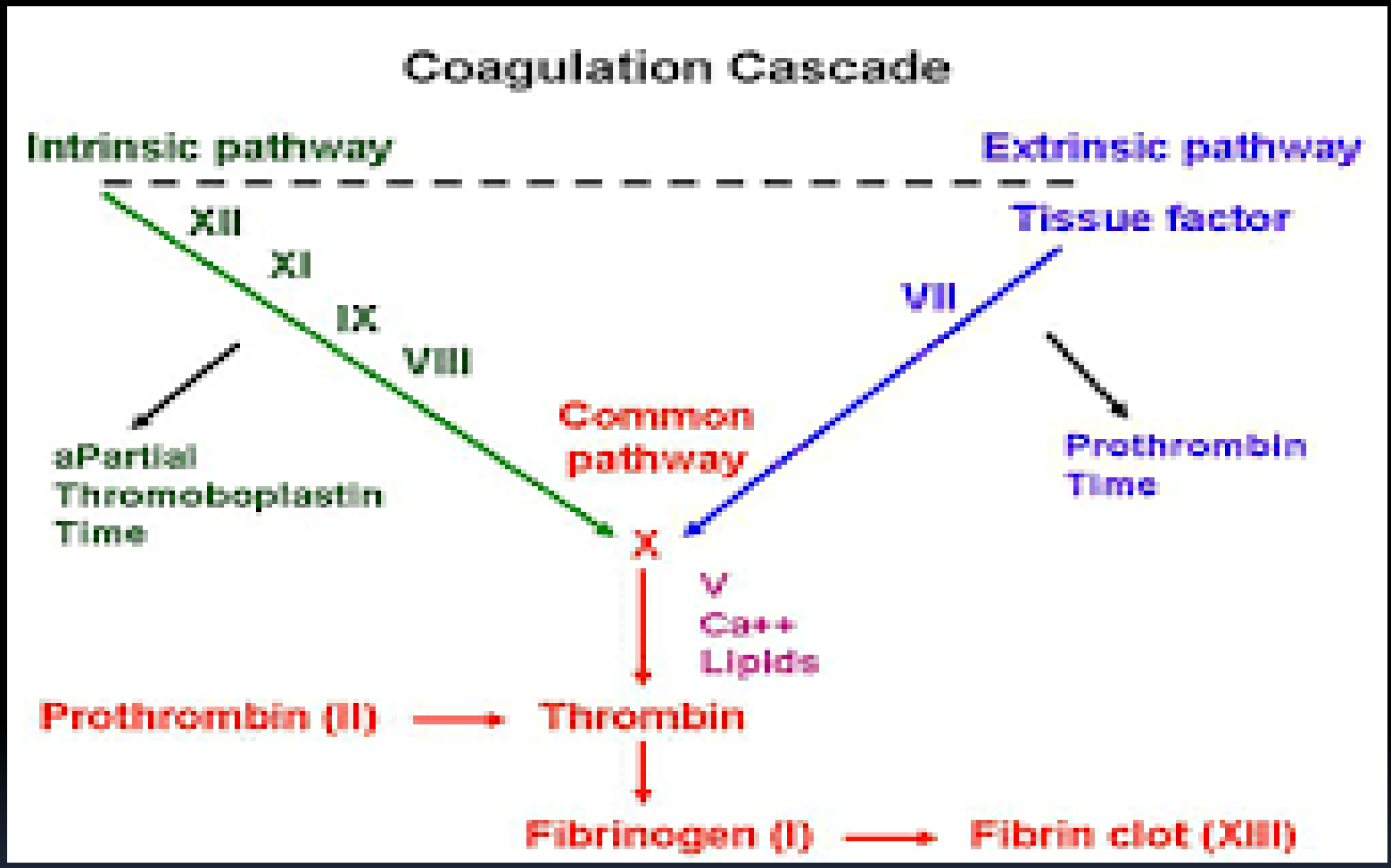
(normally 10 - 15 minutes).



It is a non specific test for

- Intrinsic
- common pathways

not sensitive to minor clotting factor deficiencies



The test time is prolonged in the deficiency of any clotting factor except factors III, VII, XIII.

2) Partial thromboplastin time (PTT) and Activated partial thromboplastin time (APTT):

These tests detect abnormalities in:

- Intrinsic
- Common pathways

a) Partial thromboplastin time: (PTT)

1) Patient's citrated plasma (i.e. deficient inCa++)

Phospholipid extract from brain tissue (partial thromboplastin without **tissue factor**)

known amount of Ca++

fibrin formation

Normal time = 60 - 85 Seconds.

b) Activated partial thromboplastin time (APTT):

In addition to the PTT procedure, kaoln (clay-like substance) is added to activate factor XIII (fibrin stabilizing factor).

Normal time = 35 - 50 Seconds.

PPT prolonged in

1. Hemophilia
2. Liver disease
3. Anti-phospholipid antibiodies
4. DIC

3) The one-stage Prothrombin time (Quick method) [PT]

"It is the time required for oxalated or citrated plasma to clot in the presence of added factor III (tissue thromboplastin), and Ca++"

Citrated plasma +
tissue thromboplastin +
Ca++ (known amounts) 37oC → clotting .

Normal value = 12 - 15 Seconds.

It measures

- The extrinsic
- Common pathways.

Thus it measures the efficiency of:

Factor VII (Stable factor) in addition to X, V, II and I

(it is not prolonged in haemophilia)

The most common causes for its prolongation are:

- Anticoagulant drugs
- Liver disease

The result is then converted into the "International Normalized Ratio" (INR)

$$\text{INR} = \frac{\text{PT of patient}}{\text{PT of control.}}$$

Normally it should be 1 or about.

No oral surgery should be attempted if INR exceeds 2.

4) Factor assays:

Method:

By adding all other factors except the one to be tested.

Results are expressed in % of concentration.

< 40% is considered abnormal.

This could be applied to all factors except fibrinogen (Factor I). Instead, it is quantified in plasma.

Normal factor I level in plasma = **200 - 400 mg/dl.**

It is Decreased in: -

1. **Advanced liver disease**
2. **Disseminated intravascular coagulation**
3. **Congenital hypofibrinogenomia**